

Giardia and *Cryptosporidium* Transport and Fate in Groundwater Systems

Project Scope

The potential exposure of humans to pathogens in potable water supplies is of significant and ongoing regulatory concern. One of the major factors affecting the likelihood and magnitude of human exposure is the transport and fate behavior of the pathogens in subsurface (groundwater) systems. Despite their known public health significance, only limited research has been performed on the subsurface transport and fate behavior of *Cryptosporidium parvum* oocysts, *Giardia muris* cysts, and microsporidium *Encephalitozoon intestinales* spores. Several current and proposed EPA regulations and guidance documents address waterborne *Cryptosporidium* and *Giardia*, while microsporidia (*Enterocytozoon* and *Septata* genera) are included on the 2005 Drinking Water Contaminant Candidate List (CCL). Additionally, *Cryptosporidium* and microsporidia have been shown to be resistant to chlorination, which is the primary water treatment method used throughout the United States. Thus, although these pathogens are often found in the aquatic environment and are of regulatory concern, the processes that control their transport and fate in soil-groundwater systems remain poorly understood.

The overall goal of this research grant is to examine the transport and fate of *Cryptosporidium parvum* oocysts, *Giardia muris* cysts, and microsporidium *Encephalitozoon intestinales* spores during subsurface infiltration (e.g., riverbank filtration, effluent recharge). The specific objectives are to:

1. Investigate processes influencing transport and fate of the target pathogens in model systems;
2. Investigate the transport and fate of target pathogens under the unsaturated conditions present during water infiltration events; and
3. Investigate the transport and fate of the target pathogens in a complex field system.

Grant Title and Principal Investigator

Giardia/Cryptosporidium Transport and Fate During Subsurface Infiltration: Integrated Laboratory and Field Study (EPA Grant #R829013)

Mark Brusseau, University of Arizona

Key Findings and Implications

Analytical Accomplishments:

- Miscible-displacement (packed column) experiments with MS2 coliphage, used as a control data set for comparison with target pathogens, showed that a large fraction of the injected viruses appeared to have been retained within the column.
- The transport behavior and recovery of microsporidium *E. intestinales* was found to be similar to MS2 coliphage.
- The recoveries for *Cryptosporidium* oocysts and *Giardia* cysts were significantly less than those observed for MS2 coliphage and microsporidium *E. intestinales*.

Implications of Research and Impacts of Results:

- This work has demonstrated that miscible-displacement experiments can be used to study transport and fate of pathogens of public health concern and in conjunction with field experiments and mathematical modeling.
- The retention of microorganisms in these packed column experiments suggests their removal through processes such as filtration and straining; however, straining appears to play a greater role on the retention of larger pathogens (e.g., *Giardia*) than for smaller pathogens.
- Accumulation of pathogens at air-water interfaces may influence their retention and transport in unsaturated porous media.

Publications to date include 1 journal article in press, 2 articles in review, and 2 conference presentations.

Project Period: September 2001 to August 2004 (no-cost extension through August 2005)

Relevance to ORD's *Drinking Water Research Multi-Year Plan (2003 Edition)*

This project contributes to all three Long-term Goals for drinking water research: (1) By 2010, develop scientifically sound data and approaches to assess and manage risks to human health posed by exposure to regulated waterborne pathogens and chemicals, including those addressed by the Arsenic, M/MDP, and Six-Year Review Rules; (2) by 2010, develop new data, innovative tools and improved technologies to support decision making by the Office of Water on the Contaminant Candidate List and other regulatory issues, and implementation of rules by states, local authorities and water utilities; and (3) By 2009, provide data, tools and technologies to support management decisions by the Office of Water, state, local authorities and utilities to protect source water and the quality of water in the distribution system.

The results of this research enhance understanding of the transport and fate of three pathogens of public health and regulatory concern, including microsporidia that is included on the current (2005) CCL and *Cryptosporidium* that is included on the proposed Long-Term 2 Enhanced Surface Water Treatment Rule. It also addresses coliphage, the monitoring of which is included in the proposed Ground Water Rule. The continued refinement and application of the miscible-displacement experiments and related field studies and mathematical modeling developed by the researchers under this grant will help improve future risk assessments and risk management of pathogen occurrence in water supplies. It will also provide information useful for evaluating the efficacy of *in-situ* filtration approaches for pathogen removal.

To carry out these objectives, the researchers have been conducting laboratory, field, and modeling experiments to characterize the processes influencing the transport and fate of these pathogens during infiltration of effluent through subsurface systems. Completed, ongoing, and planned studies for this project are summarized below.

Project Results and Implications

Much of the work under this grant is based on the use of “miscible-displacement” experimentation (i.e., the movement, mixing, and displacement of two or more soluble fluids through a porous medium). More specifically, a column is packed with a well-characterized porous medium, and solutions containing the solute of interest (nonreactive tracer, microorganisms) are pumped into the column. Effluent from the column is sampled and analyzed to obtain solute concentrations.

In the first year of this project, the researchers conducted a series of experiments with MS2 coliphage, a widely used model virus that infects *Escherichia coli* bacteria, to establish a control data set to which the microbial transport data can be compared. The results of these initial experiments showed that a large fraction (approximately 45 percent) of the injected virus traveled through the soil at the same velocity as water; that is, minimal retention was observed for a large fraction of the viruses. The remaining viruses appeared to have been retained within the packed soil through quasi-irreversible sorption mechanisms (e.g., binding to geologic materials because their surfaces are charged), and a portion may have been inactivated. However, several difficulties were encountered in the initial pathogen (*E. intestinales*) miscible-displacement experiments in the first year of this project that required an extended period of troubleshooting to resolve. For example, a major problem was that the initial supply of *E. intestinales* spores was found to be degrading before experimental use that required a change in the supplier.

In the second year of this research, a series of miscible-displacement experiments with *E. intestinales* were conducted. Preparation for the experiments began with the uniform dry packing of the column with a well-sorted, low organic matter silica sand. The column was saturated with an electrolyte solution, and pentafluorobenzoic acid was used as a conservative tracer to characterize the hydrodynamic properties of the porous medium. Solutions containing the inactivated *E. intestinales* were injected into the column, and all effluent samples were analyzed using epifluorescent microscopy. The results revealed that a large

fraction of the spores pumped through the column are not recovered in the effluent, suggesting removal through processes such as straining (i.e., when particles are larger than the largest pore diameters and cannot penetrate into the porous medium and are thus filtered at or near the surface) and attachment. Efforts to identify the specific processes responsible for this removal were conducted in the third year of the project, and are summarized below. In general, the transport behavior observed for *E. intestinales* was found to be similar to that observed for the MS2 coliphage (described previously). The researchers also started developing a mathematical model to describe pathogen transport and fate in porous media. The model was designed to incorporate multiple processes specific to pathogen transport including reversible sorption, irreversible attachment, surface blockage, straining accumulation at the air-water interface (for unsaturated conditions; see more below), and inactivation.

During the third year of the project, researchers conducted a series of miscible-displacement studies with *Cryptosporidium* oocysts and *Giardia* cysts similar to those conducted on MS2 coliphage and *E. intestinales* in the first and second years of the project, respectively. In addition, several porous media with different mean grain diameters and grain-size distributions were used to investigate the role of straining on retention of the pathogens. The recoveries for *Cryptosporidium* and *Giardia* were found to be significantly less than those observed for MS2 coliphage and microsporidia. This indicates a larger role of straining on retention of larger pathogens. Furthermore, recoveries were greater in experiments using a porous medium with a larger mean grain size than to those obtained using smaller grains. Based on these findings, additional experiments to clarify the impact of straining on pathogen retention were planned for the fourth and last year of the project. The researchers suspected that accumulation of pathogens at air-water interfaces may influence their retention and transport in unsaturated porous media. Thus, in such cases, knowledge of the magnitude of the available air-water interfacial area—although a very difficult property to quantify—is critical for predicting transport behavior for pathogens. To this end, the researchers conducted a series of gas-phase partitioning tracer experiments to measure air-water interfacial areas as a function of water content for several types of porous media. Using these data, the researchers developed a set of correlation equations that can be used to estimate air-water interfacial areas using information on specific surface area and uniformity coefficient (grain-size distribution) of the porous medium. The researchers also continued work on a mathematical model to describe pathogen transport and fate in porous media.

The researchers plan to continue the miscible-displacement experiments for the target pathogens in the fourth and last year of the project. These experiments are intended to characterize the primary mechanisms influencing the retention and transport of the pathogens. A field experiment is also planned to examine pathogen and transport under more natural conditions. The development and testing of the mathematical model will be completed and used to simulate the results obtained from the miscible-displacement experiments described above, as well as for select data sets taken from the literature.

Investigators

M. Brusseau, University of Arizona
W. Blanford, Louisiana State University
C.P. Gerba, University of Arizona

For More Information

NCER Project Abstract and Reports:

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/1068/report/0

Peer Reviewed Publications

Peng, S., and Brusseau, M.L. 2005. Impact of soil texture on air-water interfacial areas in unsaturated sandy porous media. Water Resources Research (in press).

Blanford, W.J., Brusseau, M.L., Yeh, T.C.J., Gerba, C.P., and Harvey, R. 2005. Influence of water chemistry and travel distance on bacteriophage PRD-1 transport in a sandy aquifer. Water Research (in review).

Oleen, J.K., Brusseau, M.L., Santamaria, J., Orosz-Coghlan, P., Chetochine, A., Blanford, W.J., Rykwalder, P., and Gerba, C.P. 2005. Transport of microsporidium *Encephalitozoon intestinales* spores in porous media. Water Research (in review).